A study on measurement, distribution and characterization of biochemical constituents of atmospheric aerosol over semiarid region of Indo-Gangetic Basin

A
SYNOPSIS

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1. Introduction

Atmospheric aerosols are tiny particles and ubiquitous in nature. They are found everywhere from inland to arctic and from troposphere to stratosphere. Aerosol enters into the atmosphere from different sources, affects solar radiation, causes visibility impairment; alter the atmospheric chemistry and affects human health. The effects of aerosol depend upon physical, chemical and biological characteristics of aerosol. Physical properties includes mass, number, shape and size of particles; chemical characteristics include chemical constituents of aerosol viz., major cations, major anions, hydrocarbon, organic carbon, heavy metals etc while biological constituents include bacteria, fungi, viruses, pollens, bio-films, debris etc. Biological constituents of aerosol are commonly termed as 'bioaerosol'. Primary biological aerosol particles (PBAP) such as fungal spores, bacteria, viruses, proteins and fragments of organisms are ubiquitous components in atmospheric aerosols. They can act as cloud condensation nuclei (CCN) and ice nuclei (IN); affects melting and processing activities of snowpack; metabolize organic compositions; and affects human health. The effects of biological constituents of aerosol get exacerbated in the presence of chemical constituents of aerosol particularly heavy metals. However, biological constituents of aerosol have attracted little attention of scientific community because of the complexity in measurement, composition, distribution, emission and evolution.

Once aerosol particles are in the atmosphere, transformation, transport and removal can take place on the basis of aerosol sizes, concentration and chemical and biological composition, location and meteorological conditions. Bioaerosol present in the atmosphere are bacteria, algae, protein, virus, animal dander, bio-films, microbial toxins, plant fiber and living or dead bodies,

pollens, fungal spores, fragments of plants, animals or any living organism and debris. The size range of bioaerosol is from 0.1 to 100 μm. However, most of the microorganisms except the pollen are in the fine size range (< 2.5 μm). About 25 % of the total airborne particulate matter may be made up of biological materials (Jones and Harrison, 2004). The concentration of particles depend upon the temperature, relative humidity, wind speed and anthropogenic activity viz., fuel combustion by industrial process, transportation, disposal of solid waste. They are important constituents of aerosols and affects atmospheric chemistry. Bioaerosol have significantly affected the weather and global radiative balance by scattering and absorbing radiation. The bioaerosol emitted during monsoon rainfall could potentially play important role in cloud and precipitation (Ansari et al.,2015) as they act as cloud condensing nuclei (CCN) and ice nuclei (IN) and hence affect climate and alter atmospheric chemistry (Jaenicke, 2005; Ariya and Amyot 2004; Sun and Ariya, 2006). The abundance of bioaerosol is strongly dependent on location and season but in India it is poorly characterized and need to be addressed systematically (Ansari et al., 2015). Indo-Gangetic Basin is experiencing drastic change in climate and facing increase in number of epidemic diseases in the last few decades (Negi et al., 2012). This basin is also suffering from high load of aerosols. The increase in epidemic diseases has been associated with the increase in aerosol concentration. Hence, there is an urgent need to study the biological constituents of aerosol.

1.1 **Origin and sources of bioaerosol**

Bioaerosol are originated by a variety of sources, like earth crust, natural sea spray, windblown dust, humans (skin scales), plants (pollens and spores), fungi (spores, toxins and VOC) and bacteria (cell, cell fragments, spores and toxins). Major sources of bacteria and viruses are from humans and pets-sneezing, coughing, dander and saliva. Fungi, bacteria, protozoa, algae and green plants (pollen) are present outdoors as well as indoors. Amplification sites are moist surfaces of leather, wood, carpets, soap, cloth fabric, pastes and adhesives. Breeding sites for fungi and bacteria are humidifiers, air conditioning systems, cooling towers, air distributing systems and areas of

water damage. Food stuffs, house plants and flower pots, house dust, pets and their bedding, textiles, carpets, wood material and furniture stuffing etc, occasionally release spores of *Alternaria, Aspergillus, Botrytis, Cladosporium, Penicillium, Scopulariopsis* into the air (Maeir et al., 2002). The spores having an aerodynamic diameter between 2-10 µm can access into respiratory system and affect the human health. The highest amounts and the most diverse of microorganisms are found in a domestic sewage along with animal and human solid waste disposal (Momin et al., 1999) which may include a number of bacteria (Patentalakis et al., 2008) and filamentous fungi (Kacprzak et al., 2005). The toxin produced by bioaerosol like *C. botulinum* is a potential biological warfare agent (Bioterrorism) (Sharma, 2013).

1.2 Biological constituents of aerosol

The biological constituents of aerosol are bacteria, fungi, viruses, pollens, endotoxins etc. They have potential to alter cloud, affect atmospheric chemistry and pose threats to human health.

1.2.1 Bacteria

Bacteria are ubiquitous in the near-surface atmosphere. About one billion different species of bacteria (Prokaryotic microorganism) exist in almost every ecosystem on Earth. The shape and size of bacteria varies. Bacteria are of two types Gram Positive and Gram Negative. Gram +ve bacteria have a rich peptidoglycan layer that lacks outer membrane while gram –ve bacteria have thin peptidoglycan layer on outer membrane of lipophilic polysaccharides and a phospholipids bilayer. The term Gram refers to a technique developed by Hans Christian Gram in 1884 to differentiate bacteria by staining. It depends upon water, pH, temperature, oxygen concentration, nutrient source (Stetzenbach et al., 2007). Some of the bacteria are pathogenic and some are non pathogenic. Bacteria act as CCN and also cause various diseases like tuberculosis, hypersensitivity, pneumonitis (Farmer’s lung disease).

1.2.2 Fungi

Fungi are unicellular eukaryotic organisms that lack chlorophyll and have rigid cell wall composed of chitin and glucans. Glucans are the glucose polymers that are structural components of fungi (molds and yeast), bacteria and plants (Douwes, 2005). It is estimated that 1.5 million species may exist causes toxic pneumonitis, hypersensitivity, pneumonitis, chronic fatigue syndrome, kidney failure and cancer (Prescott, 2005). The various groups of fungi are yeasts, molds and mildew. People are exposed to this mold spores or fragments through inhalation or by contact through small breaks in skin.

1.2.3 Viruses

Airborne viruses transmit much number of diseases into human being and it affects plants/crops also. Chickenpox, influenza, measles, smallpox, cryptococcosis, and tuberculosis are a few commonly known diseases caused by viruses suspended in the air.

1.3 Effects of bioaerosol on climate

Biological constituents of aerosol have been reported to be present everywhere even in the stratosphere. They act as cloud condensation nuclei (CCN), ice nuclei (IN) and alter cloud coverage and hence the global climate. They also alter the atmospheric chemistry due to microbial degradation. Bioaerosol are organic in nature. They have potential to modify the chemical composition of other organic compounds upon collision or contact. They undergo decomposition and liberate heat although it may be very less in amount. Hence, they are also responsible for global radiation. Bioaerosol induces chemical reaction and transform atmospheric dicarboxylic acid (DCA) (Ariya and Amyot, 2004). Organic compounds can also be transformed in snow by microbiological process. The CCN capabilities get affected once the size and density along with chemical composition of organics get changed. The lifetime of organic aerosols in the atmosphere

vary from days to weeks and several types of airborne taxa are attached to other aerosol such as dust or seas salt. In spite of the importance of bioaerosol, current knowledge on physics and chemistry of bioaerosol is not advanced.

1.4 Effects of bioaerosol on human health

Air borne particulates are not only responsible for visibility loss on hazy days (QUARG 1993), effects on vegetation (Pyatt, 1973) and building materials (Butlin, 1991) but they are also responsible for the increase in the incidence of human respiratory and cardiac diseases. They are also responsible for various epidemic diseases viz., rhinitis, tracheobronchitis, irritations, organic dust toxic syndrome, sinusitis, diarrhea and nausea, skin rash, itching, dermatitis, eczema, dermatomycosispycoderma etc and affects health. Bioaerosol get induced into human body by inhalation or by deposition on wounds. Despite the defense mechanism of the body these bioaerosol could cause damage to the body. The infections caused due to the bioaerosol include: legionnaire's disease, humidifier fever, cold, influenza. The presence of bioaerosol can be comprised of activities like sneezing and coughing generated respiratory droplets which transmitted several human and veterinary pathogens in hospitals, animal sheds, clean rooms, pharmaceutical facilities and spacecraft environment (Venkateswaran et al.,2003; Okafor and Opuene, 2007). Previous studies have shown the relation of particulate matter especially $PM_{2.5}$ to exacerbation of asthma, bronchitis, chronic obstructive pulmonary disease (COPD) and Lung cancer (Bonner, 2007; Li, 2015) and mortality rate increases due to lung cancer (Turner et al., 2011; Chen et al., 2015). Bacterial endotoxins are lipopolysaccharides (LPS), which are components of the outer cell membrane of gram-negative bacteria that can be released during cell lyses. They can induce strong inflammatory responses and symptoms like fever, headache, coughing, and respiratory distress (Douwes, 2003; Longhin et al., 2013). Cyanobacteria are widespread and abundant organisms in terrestrial, as well as aquatic environments, which produce neurotoxins, cytotoxins, dermatotoxins, and different types of hepatotoxins (Cox et al., 2005; Kaasalainen et al., 2012). The neurotoxins inhibit

neurotransmission by a variety of mechanisms, frequently causing death of the exposed organisms, hepatotoxins, comprising the cyclic peptide groups of microcystins and nodularins as well as the cyclic guanidine alkaloid cylindrospermin, are hepatotoxic, causing severe and sometimes toxic health effects in domestic and wild animals as well as in humans (Codd et al., 1999). WHO has declared that exposure to bioaerosol is associated with respiratory symptoms, allergies asthma and immunological reaction (Heseltine and Rosen 2009). Nosocomial transmission may occur by patients coughing or sneezing whch generates respirable particles within the range of 0.5 to 12 µm (Donaldonson and Alexanderson, 2002; Duan et al., 2008). Allergenic and toxic bioaerosol need not to be viable, as also dead cells or cell fragments may provoke the same adverse health effects. Proteins account for up to 5% of urban air particulate matter, and interactions of these proteins with ozone, nitrogen dioxide, sulphur dioxide, can lead to modified proteins with modified allergenic potential. Pathogenic fungi are a major threat to animals and plants including crops, severely jeopardizing food security (Fisher et al., 2012).

1.5 Importance of the bioaerosol study

India is a sub continent with rich biodiversity and has various geo climatic zones which marked seasonal fluctuations in temperature, relative humidity and contain nearly 18% of the world's population. Despite of their concern to human health and impact on the environment the studies related to bioaerosol are very few (Ansari et al., 2015; Valsan 2015, Mamta et al., 2016). So, there is a need of study to improve knowledge and develop modelling of the effects of bioaerosol on climate, health, and ecosystems on local, regional, and global scales and adopt control methods.

2. Literature Review

A review on biological components of aerosol has been performed. The concentration and distribution of biological constituents of aerosol have been studied but few reports are available from India.

International Status

Microorganisms are aerosolized from all surfaces like aerial plant parts, soil and water (Jones and Harrison, 2004). In recent years role of primary biogenic aerosol (PBA) fraction on human health and other ambient environmental processes has emerged as an important aerosol research area. The PBA constitutes major fraction of ambient PM load. Several studies have shown that primary biological aerosol (PBA) particles like bacteria, fungal spores, plant fragments and pollen are ubiquitous in the atmosphere which can influence atmospheric chemistry, physics and climate (Wainwright et al., 2003; Prospero et al.,2005; Despres et al.,2007, 2012).

Substantial development and application of advanced online and offline techniques for studying characteristic features of bioaerosols in both field and laboratory have been experienced in last few decades. 800 colony forming units per cubic meter are reported to be the maximum allowable value of the total bacterial bioaerosol concentration (Ministry of Environment, ROK, 2010). Several studies on the measurements of bioaerosols in outdoor and indoor environments, including schools and health care facilities were reported (Godwin and Batterman, 2007; Kim and Kim, 2007; Menetrez et al., 2007; Fang et al., 2008; Zuraimi and Than, 2008). Malecka- Adamowicz et al., 2007 have found that mould fungi were most numerous of the microbial community in the air on the premises of a landfill site. Lee et al., 2012 reported the number concentrations of total culturable bacterial bioaerosols in seven public restroom environments. Fungal spores have the ability to survive under harsh environmental conditions and they are present in ambient particulate

matter. Chehregani et al., 2004 stated that potency of fungal spores as bio-allergens increase even in the presence of smoke from automobiles and polluted air. A number of studies have reported correlation of bioaerosol load with $PM_{2.5}$ and PM_{10} concentration separately for natural desert area and an urban fringe site located in Phoenix (Borenson et al., 2004; Huffman et al., 2013). Womiloju et al., 2003 concluded that fungal cells and fragments accounted from 4-11% of the total $PM_{2.5}$ concentrations.

Biological constituents of aerosols are basically organic in nature and made up of carbon.Bauer et al.,(2002b) have described a method for organic carbon (OC) determination. Bauer et al. (2008) reported significant contributions of fungal spores to the organic carbon and to the aerosol mass balance of the urban atmosphere. The concentrations of airborne fungal spores were determined at a suburban (Schafberg) and a traffic-dominated urban site in Vienna, Austria. Fungal spores OC ranged from 22 to 677 ng m^{-3} with a summer mean value of around 350 ng m^{-3} at the suburban site and 300 ng m^{-3} at the urban traffic site. The fungal spores contributed on average 6% in spring and 14% in summer to aerosol OC mass concentration. The fungal contribution to PM_{10} was amounted to 3% and 7% at the suburban and to 1% and 4% at the urban site in spring and summer, respectively. Bauer et al., 2002a reported the OC associated with bacteria and with fungal spores in background samples collected at an alpine station. Elbert et al. (2006) reported high contributions to PM averaging 35% by weight from fungal spores in Amazonia. Fu et al., 2015 measured fluorescent water soluble organic aerosols in the high Arctic atmosphere. Organic chemistry inside clouds can also be a potentially important source of SOA (Secondary Organic Aerosol) after evaporation of cloud droplets. In fact, all bioaerosols whether pathogenic or not, allergic or not, dead or alive, are contributors to the aerosol organic carbon (OC), and need to be assessed in terms of their mass contribution to the PM aerosol.

Micro-organisms are incorporated into cloud droplets and raindrops by nucleation scavenging as they have potential to act as cloud condensation nuclei or ice nuclei (Lee et al., 2002). Amato et al., 2007 measured the concentration of ATP in cloud water indicate that most microorganisms are still metabolically active. Over the past few years, many studies have characterized the microbial content of various polar or mountain environments and the highest bacterial abundance were reported from Arctic sea ice, where concentrations can reach 1×10^5 bacteria ml^{-1} of melted ice (Brinkmeyer et al., 2003; Junge et al., 2004). 3×10^3 bacteria ml^{-1} were detected in the deep accretion ice of the sub-glacial Antarctic lake Vostok (3500 m below the surface) (Karl et al., 1999; Priscu et al., 1999; Abyzov et al., 2001). Intermediate bacterial concentration values were obtained for snow from glaciers (Skidmore et al., 2000; Zhang et al., 2002; Foght et al., 2004), polar and alpine lakes (Pearce et al., 2003), and clouds (Bauer et al., 2002a; Amato et al., 2005). Mountain snow bacterial concentrations have been reported to range from 3×10^3 cells ml^{-1} (Bauer et al., 2002a) to 4×10^5 cells ml^{-1} (Sattler et al., 2001; Segawa et al., 2005) for mid latitude environments. Amato et al., 2007 reported bacterial characteristics over the snow cover at Spitzberg, Svalbard. Bacterial degradation allow them develop when snow melts, and thus to be actively involved in snow chemistry. Ncube et al., 2017 studied bioaerosols, noise and ultraviolet radiation exposures for municipal solid waste handler. Microorganisms have potential to affect snowpack, lands and alter atmospheric chemistry

Rodo et al., 2014 reported the spatial spread of fungal spores and their biological impact. Even in the absence of location specific sources it is distributed from other location through wind assisted particulate matter transport. Several studies have reported the role of meteorology in ambient concentration of biological constituents of aerosol (Mamta et al., 2016; Kumar and Attri, 2016a). The concentration of bacteria and fungi were reported to be influenced by temperature, relative humidity, wind speed etc. Many studies have identified high levels of bacteria, fungi, and allergens in indoor environments in Taiwan. Li and Kuo (1992; 1993) indicated that temperature and relative

humidity are related to the concentration. Fluctuation in temperature can influence the pollen production (Beggs, 2004) which affects the concentration of Particulate matter (Martuzevicius et al., 2004) and ozone (Ross et al., 2002).

Particulate matter causes adverse health effect. It depends on its penetration and deposition according to their size, shape and density in the respiratory tract within human and animals (Harper, 1953; Lippmann and Albert, 1969; Schlesinger, 1985). PM_{10} (Thoracic fraction), $PM_{2.5}$ (Respiratory fraction), and fine particle (0.1 - 2.5 µm) deposit in the tracheobronchial region. Ultrafine particle < 0.1 µm particles causes the largest health threats because they can easily accumulates to the lower respiratory tract, comprising trachea, bronchi, bronchioles and alveoli and enter the blood stream (Stetzenbach et al., 2007). It can be detached more quietly from the body and thus have more possibilities to damage healthy cell tissues (USPEA, 2000). The spores having an average aerodynamic diameter between 2 and 10 µm can gain access into the respiratory system and can induce respiratory allergies, exacerbate asthma, and cause acute respiratory ailments (Pringle, 2013). Many diseases, such as tuberculosis, severe acute respiratory syndrome (SARS), and H_1N_1flu are caused by airborne microbes. The adverse effects of bioaerosols include infectious diseases, acute toxic effects, allergies, and cancer. Among these, infectious respiratory diseases are the most prevalent (Douwes et al., 2003; Lee et al., 2011).

The biological components of aerosol finally settle to the earth surface through rain or dry deposition. The estimated annual average global load of PBA in the atmosphere is~1000 Tg. Fungi is an important constituent of PBA component and contributes a significant proportion of particulate matter load (Elbert et al., 2007; Frohlich-Nowoisky et al., 2009). Dry deposition is an important mechanism of removal of pollutants from the atmosphere to earth surfaces (Hicks et al., 1991, Kumar et al., 2003; Kumar and Kumari, 2015)

The Ministry of Environment (ME) of ROK (Republic of Korea) recognize the importance of bioaerosols and regulate bioaerosol concentrations in public facilities. Air is filled with numerous tiny organisms, with sizes ranging from 50 nm to 10 μm. These organisms are called airborne biological particles or bioaerosols. The bioterrorism incidents and the many appeals were made regarding the hygiene problems associated with airborne microorganisms. The pandemic outbreak of flu due to the influenza A H_1N_1 virus in 2009 and the bio-terror incidents in 2001 have suggested the importance of bioaerosol research (Lee et al., 2012). Hence, understanding on concentration, characterization, distribution, and deposition of biological constituents of aerosol is of paramount importance.

National Status

Bioaerosols are integral part of our ecological system and play an important role in its balance. Viruses, bacteria, fungi etc. are collectively called airborne biological contaminants or Bioaerosols. Inspite of the concern on impact of biological components of aerosol on human health and environment, the characterization of bioaerosol is limited. Predominant research investigations characterizing fungal spores in different indoor environment settings (Schleibinger et al., 2005), schools (Aydogdu et al., 2005), child care centres (Zuraimi and Tham, 2008), hostels and health center (Srivastava et al., 2012), animal house and shed (Adhikari et al., 2004b; Liao et al., 2010), and food processing units (Zorman and Jersek, 2008) have reported significant concentrations and diversity of fungal spores in volume of confined air. However, similar investigations on ambient PM are limited, particularly from developing countries. In India, few investigations have reported the presence of high concentration of airborne fungal spores in suburban air (Adhikari et al., 2004a; Chakrabarti et al., 2012). A comparative analysis of culturable air borne microorganisms has been conducted in post-monsoon and monsoon season by measuring their ambient levels at four sites within Delhi premises (Lall et al., 2013). Sudharsanam et al., 2012 reported that Staphylococci and Micrococcus species were predominant Gram positive bacteria and Enterobacter and Pseudomonas

species were predominant Gram negative bacteria and *Aspergillus niger* were dominant fungi isolated from different health care setting. Mamta et al., 2015 reported the bacterial and fungal concentration is ambient air of suburban site over north central India. Valsan et al., 2015 characterized the concentration of FBAP was maximum in early morning. The measurements of fluorescent biological aerosol particle at a tropical high altitude site in southern India during the southwest monsoon season have been done (Valsan et al., 2016). Kumar and Attri, 2016b reported the 1517 spores m^{-3} fungal concentration over rural area in western Himalayan region of India. Ascomycota and Basidiomycota fungal spores were dominant. The temporal profile of the spores showed significant correlation with the ambient temperature (T), relative humidity(RH), wind speed (WS) and planetary boundary layer (PBL) height (Mamta et al., 2015; Kumar and Attri, 2016b). In rainy season, the concentration of bacteria is higher than temperate regions, which may be due to favourable climatic conditions of tropics (Kumar et al, 2013; Mamta et al., 2015). Priyamvada et al., 2017 reported the role of microbial components in aerobiology as a fraction of the primary biological aerosol particles (PBAPs). They have recorded a total of 113 macro fungal species belonging to 54 genera and 23 families.

Biological components of aerosol are reported to be responsible for allergy and asthma related hospitalization (ARH). It has been reported that airborne microbial components of aerosol enhances the allergy and infection. Pollen has been reported to be sensitive in skin-prick test (> 50%) and IgE-ELISA (Chakrabarty et al., 2016). The prevalence of *S. aureus* and *P. aeruginosa* indicated a threat to hospitalized patient and workers (Nandlal et al., 2007). It has been reported that the effects of bioaerosol get exacerbated in the presence of chemical components like heavy metals. Heavy metals sometimes act as catalyst and increase the rate of reaction of bioaerosols. It is a metallic element having high density, specific gravity or atomic rate and is toxic in nature. Heavy metal like, lead, mercury, cadmium, Arsenic, Aluminum, Nickel, Iron, Strontium, Tin etc were reported to be present in with respirable dust particles of urban air. Various sources such as combustion of fossil

fuel, metallurgical process, garbage incineration (anthropogenic) and windblown soil, dust etc (natural sources) contribute to heavy metal constituents of aerosol. They can cause cancer, neurotoxicity, immune toxicity and cardio toxicity, which affects mental functioning of brain, lower energy level, damage to blood composition, lungs, kidney, liver and other vital organs. Fungi and bacteria are found to be good adsorbed of heavy metal. Bioaccumulation or bioadsorption means an increase in the concentration of a chemical in a biological organism over time compared to the chemical concentration in the environment. Many microbes were reported to accumulate heavy metal into dilute form inside the cell structure (Kapoor and Viraghavan, 1995; Modak and Natarajan, 1996). Investigation of distribution of heavy metals in atmospheric aerosols PM_{10} had been reported (Mohammed and Namuduri, 2013; Gaur, 2013). But, simultaneous measurements of biological as well of heavy metal constituents of aerosol is lacking in spite of the fact that metals exacerbated the allergenic activity. In India, several investigators have studied on different parameters of aerosol but biochemical constituents of aerosol are least studied (Mamta et al., 2015). In the city of Taj, Agra, chemical composition and mass size distribution of atmospheric aerosols or particulate matter had been reported (Parmar et al., 2001; Kumar et al., 2006, 2015). A number of studies on wet and dry deposition of chemical components have been also done (Kumar et al., 2002, 2006, Kumar and Kumari, 2015) but none on dry deposition of biological constituents of aerosols.

OBJECTIVES

Air borne particulate matter in the recent times has become one of the biggest problems in India as they directly affect human health and directly and indirectly affects climate. The effects of aerosol depend upon the biological and chemical constituents of aerosols. Biological constituents of aerosols are popularly known as bioaerosol but they are poorly characterized. Fungal and bacterial mass contribute significantly to ambient aerosol. However, they have potential to alter the atmospheric chemistry and causes allergy to human being. The allergenic effects of biological constituents of aerosol get exacerbated in the presence of chemical constituents particularly heavy metals as they act as catalyst. It is affecting health status of residents of the cities of both developed and developing country. Although, the Indo-Gangetic basin (IGB) is heavily polluted, the measurements, characterization and deposition of biological constituents of aerosol are limited. The deposition of airborne biological components to natural surfaces, risk to human health and its role in radiative forcing is totally lacking. With this in view objectives of the present investigation are:

- to measure the concentration of PM_{10}, $PM_{2.5}$ and PM_1 airborne particles at Agra over semiarid,
- to determine the concentration of biological constituents of aerosol and its seasonal variation,
- to measure the concentration of heavy metals in representative aerosol samples,
- to determine the biomass of biological components of aerosols,
- to determine the dry deposition flux of biological components and,
- to assess the risk of biological components of aerosol on human health.

2. MATERIALS AND METHODS

2.1 Site selection and site characteristics

The study will be carried out in Agra over the Indo-Gangetic basin. Agra is located in north central India. Two thirds of its peripheral boundaries are bounded by the Thar Desert of Rajasthan and Agra is a semiarid area. The soil type is a mixture of sandy and loamy, containing excess of salts. The climate of Agra has been classified into four seasons winter (December to February), summer (March to June), monsoon (July to September) and post monsoon (October to November). The dominant wind direction is N and NW. The wind speed in Agra is mostly in the range of 1-2m s^{-1}. In Agra; sampling will be carried out at Dayalbagh, a suburban site which is 10 km away from the industrial sector of the city where due to agricultural practices vegetation predominates. The entire aerosol sample will be collected on the roof of building in the Institute campus. The sampling site lies by the side of the road that carries mixed vehicular traffic of the order of 10^5vehicles in a day and a National highway (NH-2) at a distance of 2 km south has dense vehicular traffic.

2.2 Sample collection and preparation

Atmospheric aerosol

Atmospheric aerosol samples of $PM_{10,}$ $PM_{2.5,}$ and $PM_{1.0}$ will be collected in parallel. PM_{10} and $PM_{2.5}$ samples will be collected using two Polltech fine dust samplers. It is a low volume sampler which maintains exact flow rate (16.67 L min^{-1}) during the sampling. $PM_{1.0}$ sample will be collected using Envirotech fine dust sampler at a flow rate of 10 L min^{-1}. Glass fiber filter will be used as collecting surface for PM_{10} and $PM_{2.5}$samples. Same parameter will be collected on quartz fiber filter fortnightly. Pre-desiccated, preweighed and sterile filter will be mounted in the filter holder of the sampler and sampling will be performed for 24 hours. The filter will be withdrawn after sampling for stipulated period of time and will be kept in desiccators till constant weight is

determined. The sampling will be performed twice in a week throughout the year at least for two seasons.

The mass concentration of PM_{10}, $PM_{2.5}$ and $PM_{1.0}$ in the ambient air will be determined gravimetrically. The ratio of difference in the mass of the filter before and after the sampling and total air volume (multiplication of flow rate and sampling period) will give mass concentration and can be computed as μg m^{-3}.

$$\text{Concentration } (\mu g\, m^{-3}) = \frac{\text{Mass of filter paper (after sampling} - \text{before sampling)}}{\text{Total air volume } (m^3)}$$

2.2 Biological constituents of aerosol

Filter of $PM_{10,}$ $PM_{2.5,}$ and PM_1will be divided into two parts. One half of each filter will be cut into small pieces and will be taken into 150 ml clean and sterile conical flask. 25 ml E-pure and sterile water will be poured into flask and sample will be kept on electrical shaker for 3 hours followed by on ultrasonic bath for 10 minutes. The sample will be filtered through Whatman 42 filter paper. Aqueous extract will be used for characterization of biological constituents of aerosols. The presence of bacteria and fungi will be performed using culture techniques and concentration will be expressed as cfu (colony forming unit) per cubic meter.

Concentration (cfu m^{-3}) = No. of colonies x dilution factor / total air volume (m^3)

Identification and purification of fungi and bacteria

Fungi will be cultured using Sabouraud Dextrose Agar (SDA) media. The Sabouraud Dextrose Agar (SDA) media will be prepared using peptone 10g, dextrose 40g, Agar 20g in 1000 ml distilled water and pH will be maintained to 5.6.Sufficient amount of media will be taken in petriplates and 0.1 ml aliquot of aqueous extract of aerosol samples will spread in petriplates in

laminar flow using sprit lamp. Fungi will be incubated at 27 ºC in the SDA media in BOD incubator for 3-5 days. The number of colonies developed on petriplates will be counted. The fungal spores will be identified morphologically under a light microscope using oil immersion at a magnification of 100x. Fungi will be purified by sub-culturing of desirable colonies appearing on SDA plate till pure form is obtained. A small portion of fungus colony will be taken on to a slide containing 4% of NaCl. A drop of cotton blue stain will be added over it immediately and will be left for about 1-2 minutes. The area will be covered by a cover slip and will be used for the microscopic examination. Fungi will be identified on the basis of color, presence of spores, shape, types of spore and structure. The identification of fungi will be made with the help of existing literature and laboratory manual of introductory mycology, Manual of Chrysoporium and Allied genera, a color Atlas of Pathogenic fungi (Alexopoulus and Benecke, 1962; Campbell and Johnson, 2013).

Bacteria will be characterized using Nutrient Agar media (NAM). The Nutrient Agar medium will be prepared using 1 g beef extract, 2 g yeast extract, 5 g peptone, 5 g NaCl in 1000 ml distilled water. Sufficient amount of NAM media will be taken in petriplates and 0.1 ml aliquot of aqueous extract will be spread over using sprit lamp inside the laminar flow. The plate will be incubated at 35 ºC ± 2 ºC for 1 day. The number of colony developed on petripaltes will be counted. The bacterial spores will be identified morphologically under a light microscope using oil immersion at a magnification of 100x. The concentration of bacterial spores in air will be expressed as number of spores m^{-3} air. The concentration of gram +ve and gram –ve bacteria will be done using staining techniques. The bacterial colony will be purified by re-culturing. These colonies will be streaked repeatedly on agar plates to obtain pure culture. The colony separation will be done on the basis of different colony morphology.

Biochemical characterization of microorganisms

Biochemical test will be performed to characterize bacteria present in ambient aerosol sample. The starch hydrolysis test will be performed to determine capability of bacterial colonies to use starch as carbon source (De Oliveira et al., 2007). Carbohydrate fermentation test will be performed to determine capability of bacterial isolate to use as carbohydrates. Gelatine hydrolysis test will be performed to determine capability of bacterial colonies to produce gelatinise enzyme. Degradation of gelatine indicates the presence of gelatinase enzyme (Aneja, 2003). Catalase test will be carried out by taking 2.0 ml of hydrogen peroxide on a clean glass tube. Presence of bubbles will indicate positive reaction while absence of bubble will indicate negative reaction. In indole test the formation of red colouration at the top layer will indicate positive and yellow colouration will indicate negative. Citrate utilization test will be carried out by inoculating the test organism in test tube containing Simon's citrate medium and this will inoculate for 24 hours to 48 hours. The development of deep blue colour after incubation will indicate a positive result.MR-VP test, urease test, mannitol fermentation test etc will be also used for characterization of microorganisms.

2.3 Chemical characterization of aerosol

Half part of filter paper of sample will be cut into small pieces and will be taken into conical flask. 25 ml dil. HNO_3 will be poured into the flask and sample will be extracted on ultrasonic bath for 30 minutes. Filtered aliquot will be used for heavy metal analysis. The analysis of the selected heavy metals (Zn, Pb, Ni, Fe, Mn, Cd, Cr and Cu) will be carried out using Atomic Absorption Spectrophotometer (Lab India AA8000). The sample will be stored in refrigerator till the analysis.

Protein estimation

Proteins, contained in PBAP from different sources and with distinct properties, are also known to influence atmospheric microphysics and public health. Proteins in aerosols give surrogate information about the presence of microbial communities. The protein content in aerosol samples will be estimated using Lowry's method. The different dilution of BSA (Bovine serum albumin) solution will be prepared by mixing stock BSA solution (1 mg /ml) and water in the test tube. The BSA range wills be0.05 to 1 mg ml^{-1}. A standard curve will be made from different dilutions by taking out 0.2 ml protein solution and adding 2 ml alkaline copper sulphate reagent in test tube. After mixing solution will be incubated at room temperature for 10 min. 0.2 ml folinciocalteau solution will be added to each tube and will be incubated for 30 min. Auto zero will be set in colorimeter with the blank and optical density will be measured at 660 nm. A standard curve will be plotted between absorbance and protein concentration and concentration of protein will be calculated.

2.4 Estimation of contribution of bioaerosol towards aerosol

Biomass of bacterial and fungal isolates will be obtained in broth media. Biomass assessment plays an important role in the evaluation of a particular growth factor. Biomass assessment of fungal and bacterial cultures will be performed using Sabouraud Dextrose Broth and Nutrient broth media, respectively. Test cultures will be prepared in 250 ml conical flasks containing 50 ml of the media. After autoclaving the media, bacterial and fungal isolates will be inoculated in the media. All the flasks will be kept in BOD shaker incubator for 24 hrs. at 130 RPM. The fungal and bacterial mycelium will be harvested after every 24 hrs of growth and will be separated from the culture liquid by filtration through Quartz filter. The mycelia pellet will be

repeatedly washed with distilled water and dried at 70 °C overnight. The dry weight of fungus and bacteria will be calculated as;

Dry weight = (weight of filter paper + mycelium) - (weight of filter paper)

The carbon content in bacterial and fungal biomass will be carried out using OC/EC analyzer. The fungal contribution towards total aerosol will be estimated.The carbon content of fungal spores will be calculated by multiplying the number of spores with a conversion factor of 13 pg C per spore. In this study we use spore counts and an experimentally derived factor of 13 pg C and of 33 pg fresh weight per spore for assessing quantitatively the contribution to OC and PM_{10} (Bauer et al., 2008).

2.5 Dry deposition of biological components on natural surfaces

Sampling and analysis

Dry deposition of biological components of aerosols will be measured using surface washing method. Dry deposition samples on natural surface will be collected by tagging leaves of widely distributed plants, air dried and exposing for 72 or 96 hrs. After the certain period of exposure, deposited particles will be washed off with 50 ml distilled water. Field blanks will be collected in the same manner as dry deposition to leaf surfaces by exposing the leaves for one minute.

Bacteria and fungi in dry deposition and field blank samples will be determined using culture technique as in aerosol sample. The dry deposition flux will be presented as cfu m^{-2} $d^{-1}$and deposition velocity of microbial components will be determined by dividing deposition flux (cfu m^{-2} d^{-1}) with atmospheric concentration (cfu m^{-3}).

2.6 Meteorological parameters

The meteorological parameter viz., temperature, solar radiation, relative humidity, wind speed and wind direction will be monitored using AWS-10 (M/s Rave Innovation, India) Data Logger system during the sampling period to find out the effect of meteorological condition on the variation of ambient fungal and bacterial spore.

2.7 Health risk assessment

The effects of biological components of aerosols on human health will be assessed. The data of hospital admissions of human being will be collected from OPD of the prominent hospitals of Agra. The data will be gathered and classified according to disease, gender, age, and localities. The secondary datasets obtained will be subjected for correlation analysis with concentration of microbial components to assess health risk due to bioaerosol.

2.8 Statistical analysis

Statistical analysis viz; mean, standard deviation, range, correlation analysis, factor analysis and ANOVA will be employed on the data set using SPSS 6.0 and sigma plot to see the correlation between the variable and its dependencies on each other and source will be identified.

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